GLENZ

Examination & Determination of the Sugars, Organic Acids, & Oil in the Fruit of Ampelopsis Quinquefolia

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EXAMINATION AND DETERMINATION OF THE SUGARS, ORGANIC ACIDS, AND OIL IN THE FRUIT OF AMPELOPSIS QUINQUEFOLIA

BY

EDWARD ANTON GLENZ

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Edward Anton Glenz

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IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor of Science in Chemical Engineering

Geo. D. Beal
Instructor in Charge

APPROVED: W. A. Noyes

HEAD OF DEPARTMENT OF Chemistry

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EXAMINATION AND DETERMINATION OF THE SUGARS, ORGANIC ACIDS, AND OIL IN THE FRUIT OF AMPELOPSIS QUINQUEFOLIA

INTRODUCTORY

The climbing woody vine, Ampelopsis quinquefolia, is found in woods and thickets, in Quebec to Manitoba, Cuba, Mexico, and in the United States (throughtout the central portion). It is known as the Virginia creeper, American Ivy, Five-leafed Ivy, Woodbine, and sometimes as False Grape. It bears fruit, small blue berries, usually 2-4 seeded, the water extract of which is acid and red colored. The plant belongs to the Grape family (Vitaceae) and from this, one might expect a gross resemblance in constitution between it and the grape seed which comes from the plant Vitis bicolor. The fruit of Ampelopsis quinquefolia is not edible, altho as yet it is not given as poisenous. The fluid extract of the leaves has medicinal uses.

Method of Analysis:- Both the method of Parson and that of Dragendorf for plant analysis was studied and then it was decided to use the method of Parson. On October 1, we gathered about 400 grams, air dry, of the berries and allowed them to dry. After about three weeks we began the systematic extraction of the crush-

ed fruit. An outline of this is given below.

A- Benzene extract of crushed fruit.

B- Methyl alcohol (sp. gr. .848) extract of residue.A

C- Cold water extract of residue.B

D- Dilute acid extract of residue.C

E- Dilute alkali extract of residue.D

F- Bromine treatment of residue.E

A- Benzene:- 100.6 grams of air dry berries were thoroughly crushed and subjected to a benzene extraction in a Soxhlets appratus. The extraction was continued for 8 hours. The extract was of a greenish brown color and had only a slight odor of benzene, being covered up by odors of dissolved substances.

Weight of berries taken (air dry)	100.6 grams
Weight of residue	68.1 grams
Weight of amount extracted	32.5 grams

Solution may contain, volatile oils, camphors, volatile alkaloids, glucosides, organic acids, resins, chlorophyll, fixed oils, (castor oils) fats, wax.

After evaporating off the benzene we obtained a residue, oily and dark in color and which after reaching constant weight, weighed 28.9105 grams.

Weight of dish	56.9035	grams
Weight of dish + substance	85.8140	"
Weight of extract	28.9105	"

Above we see that the amount extracted as calculated by difference is 32.5 grams. The difference probably is due to moisture, readily volatile matter, etc. The extract contained a yellow oil and a somewhat waxy substance. A portion of this was treated as

follows:- 21.5105 grams were warmed with pet. ether (B.p. 25-40) and then filtered thru tared gooch crucibles. Considerable difficulty was encountered in filtering due to the formation of a waxy ppt. on the filter paper and hence stopping filteration. However after drying and weighing we obtained .0848 grams residue.

Volatile matter etc:- After evaporating off the benzene, a portion of the extract was treated with water and the water evaporated at 110° C.

Weight of dish + benzene extract	64.2115	grams
Weight of dish	56.9035	"
Weight of extract	7.3080	"
Weight of dish + benzene after adding H_2O and drying at 110° C	64.1750	"
Loss(due to volatile oil) etc.	.0365	"

$(.0365 \div 7.3080) \times 100 = .50\%$

To the residue we added a moderate amount of warm H_2O and allowed to cool, then filtered thru fine paper, S.&S. #595.

Water solution:- The solution obtained was of a pale yellow color, tasteless and neutral to phenolphthalein. It was boiled down and brought to a volume of 100ccm. This was then divided into two parts, 1 and 2.

1:- Solution was slightly diluted, brought to boiling, and kept at about 90° C and hot $CaCl_2$ solution added. Upon standing 24 hours no ppt. formed. No ppt. appeared after standing four days.

2:- The remaining fifty cc. of solution were placed into a tared crucible and carefully evaporated upon a hot water bath. The residue was of a yellowish brown color and appeared to peel off the crucible.

Weight of crucible	42.5192	grams

Weight of crucible + residue	42.5355 grams
Weight of residue	.0163 "
Weight of residue in water extract	.0326 "

The residue was then carefully burned off and only a slight amount of substance, white in color, remained. This formed a circle around the inside of the crucible, about half way up. Weight obtained .0106 grams, = .14%. Ash is neutral to phenolphthalein.

B- Methyl Alcohol:- The 68.1 grams remaining after the benzene extraction, after drying, were extracted with methyl alcohol(sp. gr..848). The extract was of a red-blue or purple color(like that of grape juice) and contained dissolved in it a quantity of green solid matter, which separated out on cooling. Instead of following Parson's method of analysis for this extract, this was later used for organic acid determination.

Total weight residue used	68.1 grams
Total weight residue remaining	47.0 "
Total weight extracted	21.1 "

C- Cold Water:- The residue after the alcohol extraction, we dried and extracted with cold water for 16 hours. After filtering and drying, the residue weighted 45.2 grams, leaving (47.0-45.2) 1.8 grams extracted. The extract was neutral and red in color.

D- Dilute Acid:- The 45.2 grams of residue were dried and extracted with dilute H_2SO_4 (500ccH_2O-5cc conc. H_2SO_4). On testing for starch, by treating a drop of the solution with I_2, none was found. After boiling, and again testing none was found. After allowing to stand for 2 days the solution was filtered and a syripy reddish purple liquid was obtained. The residue upon wash-

ing and drying weighed 30.5 grams. Total amount extracted(45.2-30.5) 14.7 grams.

E- Dilute Alkali:- The 30.5 grams of dried residue were next treated with 500cc of a 2% alkali solution, and boiled for two hours. A black red solution was formed and this had the odor of alkali acting on wood. The solution was filtered and the residue, dried. It was then washed five times with water containing NH_4OH (2cc per 200cc H_2O) and boiled each time. The liquid, reddish-brown in color, was filtered off and the residue used for the determination of cellulose. However the bromination method did not seem to work.

SUGARS

1- Extraction 2- Identification 3- Quantitative determination.

Extraction:- 100 grams of the fresh berries were put into a 500 cc flask and covered with 300 cc of 95% alcohol. The flask was shaken throughly and carefully stoppered and set aside for five months, taking care to shake the flask 2 or 3 times a week at least. It was then taken up and after attaching a reflux condenser, the contents were boiled for 2 hours, on a water bath, the flask removed while quite warm, and the warm contents filtered, washed once with 50 cc of alcohol and then the residue again washed into the flask. To the residue 300 cc of fresh alcohol was added and the process repeated. This was carried out 4 times, the last alcoholic extract being free from color. The alcohol was then carefully evaporated off under vacuum and the residue, now of a syrup consistency was taken up with water. This was washed with 50 cc of ether, washing twice and then warmed on a water bath until all ether was evaporated off. Volume now was 500 cc. The ether removed most of the oily substance which was of a dark green color. To the water solution, which was a cherry red, lead subacetate was added until no further precipitate formed, and to this was added a little alumina cream. The ppt. was allowed to settle and the liquid filtered off into a 2 liter flask. After washing the ppt. 6 times with water, using 100 cc each time, the volume was made up

to 2000 ccm at 20°C. The liquid was perfectly clear. The residual lead salts were set aside covered with water for further examination under the heading "Organic Acids."

Identification and Quantitative determination:- Reducing Sugars as dextrose, according to Defren. The Fehling solution was prepared as follows:

(a) 34.64 grams of copper sulphate were dissolved in water, .5 cc strong sulphuric acid added, and the whole made up to 500 cc.

(b) 178 grams of sodium potassium tartrate and 50 grams of sodium hydroxide were dissolved in water and diluted to 500 cc.

15 cc of each of the above were put into an Erlynmeyer flask having a capacity of 250 cc and 50 cc of freshly boiled distilled water added. The whole was placed in a boiling water bath for five minutes; then 25 cc of the solution added from a burette, and the mixture allowed to stand in boiling water bath for fifteen minutes. The flask was then removed and the contents filtered at once thru prepared asbestos in a Gooch crucible, washed with boiling distilled water until filtrate is no longer alkaline and then finished washing with alcohol followed by ether using suction. The Gooch was then placed in a boiling water oven and dried 20 minutes, placed in a desicator and weighed as Cu_2O.

Weight of Gooch + Cu_2O	15.7715 grams
Weight of Gooch	15.5635 "
Weight of Cu_2O	.2080 "

Hence we obtain

.208 grams=208 mg.Cu_2O from 25 cc of solution. Also 208 mg. Cu_2O equal 277 mg. CuO_2 which in turn are equivalent to 124.7 mg. dextrose.

∴ we have 124.7 mg. dextrose in 25 cc,

or 4x124.7 mg. in 100 cc of solution.

Our solution originally contains the sugar extract from 100 grams of fresh berries in 2000 cc, which is equivalent to 5 grams per 100 cc. ∴ the percent reducing sugars calculated as dextrose is equal to

$$\frac{4 \times 124.7}{5.00} \times 100 = 9.97\%. \qquad (D)$$

The figures are taken from: "Defren's Table for Dextrose, Maltrose, and Levulose." It will be observed a little later that our original solution contains about .65% sugars, and of this about .5% are reducing sugars.

Reducing Sugars after hydrolysis:- 50 cc of the original solution were taken out by means of a burette, and 25 cc H_2O added, the mixture stirred, adding slowly 5 cc of conc. HCl. The flask was then placed in a water bath kept at 70° C. The temperature of the solution in the flask reached 70° in 3 minutes and was then kept at a temperature varying between 68° and 71° for 7 minutes more. It was then removed and the solution cooled, neutralized to phenolphthalein (one drop), and brought to 100 cc at 20° C. The Fehling solution was prepared just exactly as above, as nearly as possible, and 50 cc (25 cc of original) of this 100 added. It was allowed to remain on the water bath 15 minutes, filtered, washed and dried as before.

Note. We find, on writing up this work, that we have made an error in the determination of reducing sugar after enversion, by the use of 50 cc of sugar solution instead of the 25 cc called for. This gives us a greater dilution of the Fehling solution, and an accompanying slight decrease in the amount of copper reduced by the sugar.

Weight of Gooch + Cu_2O	16.0270 grams.
Weight of Gooch	15.7715 " .
Weight of Cu_2O	.2555 " .

.2555 grams Cu_2O=255.5 mg Cu_2O from 25 cc solution after inversion. Also, before inversion 25 cc solution yielded 208 mg Cu_2O. Hence the difference is due to sucrose. However, for the present, let us give the per cent reducing sugars after hydrolysis.

255.5 mg. Cu_2O=339.8 mg CuO_2 which is equivalent to 153.7 mg. dextrose in 25 cc.

∴ per cent reducing sugars calculated as dextrose(Defren's Tables) is

$$\frac{4x153.7}{5000} x100=12.29\%.$$

Sucrose:- Reduction Method:- Having found the percent reducing sugar as dextrose before and after hydrolysis, the percentage of sucrose admits of calculation. We found that the difference between the number of mg. of Cu_2O obtained before hydrolysis (208.0) and after hydrolysis (255.5) is 47.5 mg. Cu_2O. 47.5 mg Cu_2O were, therefore, reduced by the reducing sugars obtained from the inversion of sucrose.

47.5 mg Cu_2O = 63.2 mg CuO_2, is equivalent to 27.82 mg dextrose.

$$.. \frac{4x27.82}{5000} x 100=2.26\% \text{ sucrose.}$$

Clerget's Method:- The original solution, containing the extract from 100 grams fruit in 2000 cc, was again used. A 200 mm polariscope tube was thoroughly cleaned and washed with the solution to be polarized. It was then carefully filled with the solution at a temperature of 20° C when poured into it. A reading was made immediately so that after ten trials we obtained the following

-.55 Ventzke at 28°C.

Now 50 cc of the original solution were hydrolyzed as was described previously and the reading obtained, after cooling to 20° C and waiting until the thermometer alongside the tube read 28° C, was

-1.10 Ventzke at 28° C.

Since our solution contains extract from 5 grams of fruit per 100 cc, we would expect, (approximately) that for 26 grams of fruit in 100 cc we would get a reading of

$$\frac{26x-.55}{5} = -2.86 \text{ Ventzke.}$$

Also after inversion, it would read

$$\frac{26x-1.10}{5} = -5.72 \text{ Ventzke.}$$

Therefore, according to Clerget's formuls we obtain

$$S = \text{percent sucrose} = \frac{100(-2.86+5.72)}{142.66-14} = 2.22\%.$$

Thus it checks well with the grairmetric method. Since this method works well for the determination of sugar(sucrose) in the presence of any other sugar, it seems to indicate that the method of reducing sugars calculated to dextrose according to Defren is also a reliable method for determining secrose in the presence of at least levulose, dextrose, and sucrose. It is shown below that both dextrose and levulose are present.

Levulose and Dextrose:- A pure sucrose solution polarizes at 100 on the Ventzke scale at 20° C and, will after hydrolysis, polarize about -33 on the same scale at the same temperature. It is this change of rotation from right to left, which gave rise to the term "invert sugar" for the products of inversion, which are equal parts of dextrose and levulose. However, the rotation of levulose increases with the temperature, the reading -33 Ventzke being true only for 20° C. An increase in temperature of 1° causes a decrease of practically 0.5 degree Ventzke in the levorotation of the invert

sugar, and at 87°C the reading of invert sugar becomes 0°. It is this property that enables us to calculate the percent levulose in a sample of sugar. Thus, if dextrose and sucrose are also present, by polarizing the solution at different temperatures, a means of calculating the percent levulose is obtained. Thus we polarized our original solution, after it had been concentrated, at two different temperatures. Fresh 400 cc of the original solution(containing the extract from 20 grams of fruit) were concentrated by spontaneous evaporation to 100 cc(alittle less). The solution was filtered, filter paper washed, and the liquid brought to 100 cc at 20°C. Thus this liquid was four times as concentrated as the original. A 200 mm polariscope tube was cleaned and washed with a portion of the liquid and then filled and polarized at 20°C.

V= reading at 20°C = -2.40 Ventzke

V'=reading at 60°C = -0.40 "

W =weight of substance per 100 cc = 20 grams.

t =difference in temperature = 40 .

Hence from the formula

$$l=\text{percent levulose}=\frac{(v-v')100}{w(tx-0.0723)}$$, we obtain

$$l=\frac{(-2.40-.40)100}{20(40x-0.0323} =7.74\% \text{ levulose.}$$

Now knowing the percent levulose and the reducing power of the solution, we can calculate the percent of dextrose, from the formula

$$d=D-0.91$$

where d = percent dextrose

D = percent of reducing sugar as dextrose

l = percent of levulose.

d= 9.97-0.9x7.74

d= 3.00% dextrose.

It must be remembered, however, that in our determination of reducing power, we did not obtain it by Allihans method as is advised for the correct usuage of this formula.

Osazone Formations.

A portion of the sugar solution was concentrated on the water bath and brought to such a volume that it contained .2 gram of sugar(mixture) per 5 cc and to this .4 gram of phenylhydrazine and .6 gram sodium acetate was added, and the mixture placed in a water bath. An osazone appeared after 10-15 minutes and after purifying, was found to melt at 194°C(Corr). The heating, however, was prolonged. Upon repeating upon another sample, we obtained a melting point of 204°C, after heating so as to reach 200° C in 3 minutes. From this I should think that fructose or glucose was producing the osazone.

ORGANIC ACIDS

(a) Extraction (b) Identification (c) Quantitative determination

Extraction:- The residue, left after the benzene extract, (see page), was carefully extracted as described under "Alcohol Extraction" page 4. The methyl alcohol solution thus obtained was concentrated until the odor of alcohol disappeared. The residue was taken up with distilled water, about 150 cc, and to the solution basic lead acetate was added, drop by drop until no further ppt. formed. To this mixture alumina cream was added in quantity equal to twice the volume of basic $Pb(C_2H_3O_2)_2$ used. The whole was allowed to stand when the ppt. settled and the liquid was filtered. The resulting lead salts were washed and finally rinsed into an Erlynmeyer flask and decomposed with H_2S. Before filtering the remaining ppt. was allowed to settle thus taking the oil, as well as a good portion of insoluble flocculent material, down with it. The liquid was of an orange color, and after boiling out the excess H_2S, was clear and had a volume of 100 cc. The solution now may contain any organic acids, which are soluble in water. From here we followed the method of Barfold, for the separation of oxalic, tartaric, citric and malic acid. However, it was soon learned that the presence of tannic acid and tannins interfered seriously, and hence it was decided to begin over again.

1868-Zeitsch Anal. Chemie 7, 408.

The second time, 50 grams of the air dry fruit were digested in a 2% HCl solution for one hour, boiling the mixture constantly. The solution was then filtered, and after thoroughly washing was concentrated to 200 cc. It was then boiled with very finely powdered charcoal and after filtering and thoroughly washing the charcoal, a very pale yellow filtrate was obtained. This filtrate, it is evident, contains all the organic acids, sugars, and solution of metals which are in the fruit. That calcium was in solution, as chloride, was evident from the flame coloration obtained on holding a platinum wire, which had previously been dipped in the solution, in a flame. After concentrating the filtrate to 200 cc, it was cooled and neutralized with NH_4OH solution and a ppt. formed immediately. It was a bulky ppt. almost white in color, and in general had the appearance of the ppt. of calcium oxalate and tartrate, as obtained from a pure solution of these acids. The ppt. was carefully dried and weighed (100° C).

Weight of dish	10.4650	grams
Weight of dish + Ca salts	11.6050	"
Weight of Calcium + salts ppt.	1.1400	"

Thus we obtain 1.1400 grams of calcium oxalate plus calcium tartrate plus the calcium salts of any other organic acids present whose calcium salts are insoluble in alkaline solution. Sulphates were previously removed and altho the test for phosphoric acid was positive only a trace was indicated and hence no separation made.

The ppt. was next washed into a beaker and a solution of strong NaOH was added. After digesting in the cold for 2 hours, the solution was filtered and the residue weighed. The filtrate was boiled; a ppt. formed, indicating tartrate. The residue, after drying

weighed .9000 grams and calculating as CaC_2O_4 - (pure) we obtain therefrom .605 grams oxalic acid(pure) or

$$\frac{.605 \times 2 \times 100}{100} = 1.21\% \text{ of the air dry fruit.}$$

The residue obtained on boiling did not produce a mirror by reducing $AgNO_3$ in ammonia solution, but a reduction of the nitrate did take place, leaving a gray mass at the bottom of the tube. Hence only an indication of tartaric acid exists. The difference between the weights of ppt. obtained is .2400 grams and this is probably all a reducing material. Whether it is tartaric acid or not remains to be proved. In each case the ppt. gave the calcium flame coloration. Hence, it is evident that enough calcium was in solution to precipitate the oxalic, tartaric,etc.,acids present.

The filtrate as obtained above was concentrated, cooled and made slightly alkaline with NH_4OH. To this $CaCl_2$ was added in excess and no ppt. formed. Then 4 times the volume of methyl alcohol was added and a bulky white ppt. formed, similar to a ppt. obtained on treating malic and citric acids under similar conditions The solution was filtered, the filtrate containing all soluble calcium salts of organic acids. After thoroughly washing the ppt. with alcohol, the residue was dried and weighed.

Weight of dish + Ca salts	18.5321 grams
Weight of dish	16.4321 "
Weight of Ca salts	2.1000 "

Thus we have 2.1000 grams of Calcium salts of organic acids which are soluble on adding alcohol. The dried precipitate was tested both for citric and malic acids. First, to 1.05 grams of the calcium salts, we added water, and, after dissolving with a little HCl, and again neutralizing with NH_3, we boiled for some time.

After a few minutes a ppt. formed which aggregated into flocculent, rather fine, flocks and settled to the bottom. We filtered and boiled again, but obtained no further ppt. The dried residue weighed .6100 grams. This was put into a test tube, a solution of H_2SO_4 added and then a few drops of $K_2Cr_2O_7$. The mixture was boiled and the liquid turned to a yellowish green color indicating citric, or citric and succinic acids. Calculating that only citric acid was present, we obtain the following result:-

$$\frac{.6100 \times 2 \times 210 \times 100}{100 \times 504} = .58\% \text{ citric acid } C_6H_8O_7 \cdot H_2O$$

To the filtrate, which may contain malic acid we added a little HCl and then a few drops of $K_2Cr_2O_7$ and boiled. The solution turned green but the apple odor was not distinct. The test was repeated but again no distinct apple odor was obtained. Here again further investigation is necessary since so many other organic acids form salts, with calcium, that are soluble in water but ppt. with alcohol etc. especially succinic acid. Hence as a generalization, we might say that positive tests were obtained for tannic acid, oxalic acid(1.21%) and citric acid(.58%), and a very good indication of tartaric acid indicating quite a bit of tartrate, and a fair indication of malic acid.

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OIL

1- Extraction 2- Properties 3- Physical constants 4- Chemical constants 5- Fatty acids.

The air dried fruit was crushed between two pieces of board and 100 grams of clean seeds picked out. These were washed with low boiling (25°C-40°C) petroleum ether, the washings saved, and the seeds crushed in a porcelain mortar. The extraction was then carried out in a Soxhlet extractor. After extracting for 8 hours the seeds were dried and ground to a fine powder. This was further extracted for another 8 hours. The final volume of extract was 150 cc.

The extract was of a yellow color and appeared very oily. The petroleum ether was evaporated down to about 100 cc on the water bath, and then further evaporated under an atmosphere of CO_2. The resulting oil was placed in a vacuum dessicator and kept therein for 3 days, when only a slight odor of petroleum ether prevailed.

Weight of flask(after drying in water oven)	34.4490 grams
Weight of flask + Oil(after drying in water oven)	60.0520 grams
Weight of Oil	25.6030 grams

Equivalent to 25.60% of seeds

The oil looked very much like olive oil, perhaps a little more yellow, was odorless and had no particularly definite taste altho it resembled castor oil to a certain extent. The oil was

perfectly homogeneous, no individual layers separating, on standing. After standing for about 6 days some solid fatty matter separated. On keeping the oil in a bath of ice and water, it changed to a pasty state, and when the temperature of the bath was lowered, it, the oil, solidified to a solid mass resembling solidified oleic acid. The oil gave the elaidin reaction, producing a white solid and a brown liquid. The latter may be due to NO_2 fumes absorbed, but I think not. On exposing a few drops of the oil, spread in a very thin layer, a colorless film formed in about 10 to 14 days. It was found that the oil absorbed oxygen from the air at the following rates:

1 st 70 hours	.0011 grams	equivalent to	.99%	sample
2 nd 70 hours	.0037 "	equivalent to	3.34%	sample

However the oil did not form a film for at least 7 days.

Saponification:- The saponification value indicates the number of milligrams of KOH required for the complete saponification of one gram of a fat or wax; in other words, it represents the amount of KOH, expressed in tenths percent, requisite to neutralize the total fatty acids in one gram of a fat or wax(Lewkowitsch).

Definite quantities of purified and filtered samples were weighed into flasks of about 200 cc capacity and 50 cc of an alcoholic potash solution(prepared as per directions on page 144, "Sherman Organic Analysis") added. The mixture was allowed to stand over night and then boiled for three hours. At the same time the saponification of olive oil was carried on to serve as a possible check.

	Oil		Olive Oil	
	#1	#2	#1	#2
Weight of flask(grams)	43.0111	43.1011	58.1917	58.4080
Weight of flask + sample	46.5580	47.7502	62.0440	64.0085
Weight of sample	3.5469	4.6491	3.8523	5.6005
Blank requires	61.64 cc .5165 N HCl		61.18 cc .5165NHCl	
No. cc required to neutralize	38.17	30.64	34.64	23.21
No. cc used up	23.47	31.00	26.56	37.97
Saponification number	193*	194	199	197

$$^{*}\frac{23.47 \times 56.16 \times .5165 \times 1000}{1000 \times 3.5469} = 193.$$

The titration was carried out in cold and immediately after saponification, phenolphthalein being used as indicator.

Iodine Number:- The (bromine or) iodine value indicates the percentage of (bromine or) iodine absorbed by a fat or wax, expressed in terms of (bromine or) iodine. (Lewkowitsch)

The oil was carefully weighed out into ground glass stopper flasks and 10 cc of $CHCl_3$ added. The oil dissolved quickly and then 25 cc of iodine solution were added. The whole was allowed to stand in a cool place for 1/2 hour. Upon removing 20 cc of a 15% solution of KI were added and the mixture well shaken. Then 100 cc of distilled water(air free) were added. This was then titrated with standard $Na_2S_2O_3$. Again olive oil was run as a check.

	OIL		Olive Oil	
	#1	#2	#1	#2
Weight of flask(grams)	68.5919	68.6447	68.6434	68.5919
Weight of flask + sample	68.8972	68.9703	69.0390	69.3710
Weight of flask	.3053	.3256	.3956	.7791
Blank requires	37.01 cc $Na_2S_2O_3$(.0938)		37.05 cc $Na_2S_2O_3$	

	Oil #1	Oil #2	Olive oil #1	Olive oil #2
No. cc required to neutralize	13.77	12.30	11.3	8.1
NO. cc used up	23.24	24.71	25.75	28.95
Iodine Number	90.58*	90.30	78	44

$$^{*}\frac{23.24 \times .12685 \times .0938 \times 100}{.3053} = 90.58$$

Here it is quite evident that 1/2 hour is too short a time for the complete reaction to take place when using .7791 grams oil. However it is noticed that with .3956 grams of olive oil we obtained an iodine value of 78 which is very close to that given by Lewkowitsch and hence we can reasonably assume that we alloted enough time to our determination.

Acid value:- The acid value indicates the number of milligrams of KOH required to saturate the free fatty acids in one gram of a fat or wax; or in other words, it gives the amount of KOH, expressed in tenths percent, necessary to neutralize the free fatty acids in a fat or wax. (Lewkowitsch)

One gram of the oil was thoroughly washed with water and after drying .5702 gram of the sample was dissolved in methyl alcohol. phenolphthalein being used as indicator.

Number of cc 2/10 alkali (aqueous) used for blank .32

Number of cc 2/10 alkali (aqueous used for sample .27

From this it appears as though the oil instead of being acid is slightly askaline. I did not have enough sample to repeat, but if the determination was correct, presence of basic compounds is indicated.

Unsaponifiable Matter:- By unsaponifiable matter is meant all of those substances which are insoluble in water, or do not combine with caustic potash to form soaps. (see Lewkowitsch) 4.2900 grams of oil were completely saponified. The solution was acid-

ified and allowed to stand for a few days, after which it was made neutral to phenolphthalein. The bulk of the alcohol was evaporated off and the residual soap dissolved in hot water. The solution was then made neutral again and left slightly pink. Ether was added and a good extraction made. After washing the ether, it was run into a tared dish, evaporated, and the residue dried and weighed.

Weight of dish + unsaponifiable matter	41.8900	grams.
Weight of dish	41.8178	" .
Weight of unsaponifiable matter	.0722	" .

Equivalent to (.0722 ÷ 4.2900) x100 = 1.67%.

Most all oils and fats contain in their neutral state small quantities of unsaponifiable matter, which consists, to a large extent, of

phytosterol - in vegetable oils, and
cholesterol - in animal oils.

Microscopic examination:- The unsaponifiable matter obtained above was taken up with ether, the ether evaporated off in the air, the extract dried at 100°C for one hour and then dissolved in absolute alcohol. An examination was made, but unfortunately no crystals were obtained. This, however, does not prove that phytosterol was not present, on the contrary I think that the small amount of material and the presence of some oil together with coloring matter prevented crystallization. However, the residue was taken up in ether again, and dried as before, and then treated with 3 cc acetic anhydride. I used 3 cc because I had so little material that I had difficulty in keeping the anhydride and substance mixed. The dish was placed over a small flame and allowed to remain so until the solution boiled, keeping the dish covered with a watch glass. When the solution boiled steadily, the cover glass was removed, and the excess of acetic anhydride evaporated

on the water bath. The contents of the dish were dissolved in the smallest possible quantity of absolute alcohol and then set aside to crystallize. We obtained two batches of crystals, and upon comparing them with crystals obtained by treating the unsaponifiable matter from olive oil in identically the same way, it was observed that the two were practically the same. A few of the crystals were gathered washed carefully with 95% alcohol and a melting point determined. This, it was found did not coincide closely with that of phytosterol acetate as is shown below.

Phytosterol acetate	127°C - melting point
Cholesterol acetate	114°C - " "
Acetate obtained	120°C - " "

As far as these results indicate, one could possibly assume phytosterol to be present, but let it be understood that in our case, no crystals melting at 127°C or thereabout were obtained. Hence it cannot be conclusively said that phytosterol is present. Unfortunately the time was very short and the quantity of material very limited, hence the work was not carried on beyond the above stage.

The ether extraction of the aqueous soap solution was the method employed thruout. As a suggestion, I would say that in repeating the above work, 10 grams of pure oil be used, the saponification carried out 6 hours with alc. KOH, and that the first ether extraction, after alc. is evaporated off, be made on a slightly alkaline solution. Then neutralize with acetic acid until the solution is just colorless, and complete extraction, with ether, thereby preventing the formation of emulsions.

Specific Gravity:- A small pycnometer was thoroughly dried, filled with distilled water and placed in a water bath, which was

carefully regulated to remain between 15° and 15.5°C. After one hour it was removed, and after thoroughly drying, weighed, using

.4900 grams

.3908 "

.4008 "

illed with

. After

.4800 grams

4.9900 "

$\frac{.9900}{.4008}$ = .9239

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Five readings

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small flame, it was observed that it did not decompose up to 100° C.

TABULATED CONSTANTS AND VARIABLES OF THE OIL AND its Fatty Acids

OIL	Source	Native Country	Yield from seed	Specific Gravity	Melting Point	Insoluble Fatty Acids & unsaponifiable matter	Saponification Value	Iodine value	Refractive Index	Acetyl value	Unsaponifiable matter	Mixed Fatty Acids: % in oil	Mixed Fatty Acids: Specific Gravity	Mixed Fatty Acids: Neutralization value	Mixed Fatty Acids: Iodine value	Mixed Fatty Acids: Acetyl value	Mixed Fatty Acids: Mean molecular weight	Liquid Fatty Acids: % in oil	Liquid Fatty Acids: Neutralization value	Liquid Fatty Acids: Mean molecular weight	Liquid Fatty Acids: Refractive Index	Liquid Fatty Acids: Iodine value	Solid Fatty Acids: % in oil	Solid Fatty Acids: Iodine value
CASTOR	Ricinus Communis	East Indies	46.53	.966	liq	------	183 186	83 86	14799	149 150	.55	--	.9509	192	87 93	---	293	---	---	---	---	106	---	---
GRAPE SEED	Vitis Vinivera	Asia	10.20	.925	liq	92.17	178	96	14713	---	---	-	----	197	92	---	[illegible]	---	---	---	---	151	---	---
CROTON	Croton Tiglium	East India	53.56	.95	liq	89.0	210 215	102 104	----	---	-	--	--	201	111	---	279	----	---	---	---	---	---	---
AMPELOPSIS QUINQUEFOLIA	Ampelopsis quinquefolia	Tropical countries United States	25	.924	liq	96.0	186 194	86. 90	14762	36 (cal)	1.867	96	----	203	90[2] 102[2] 96[4]	42.9	275	84.5	199[1] 170[3]	292[1] 330	14702	103 125[2] 130	1.4	53[1] 59[2]

1-Values given with this notation are those obtained using Gusserow's method of separation.
2-Values " " " " " " " " Torellis and Ruggeris method.
3-This value is the neutralization value of the liquid fatty acids whose lead salts are soluble in ether and Pet. ether.
4-This is the value as calculated from the values of liquid and solid fatty acids obtained according to Gusserow.

carefully regulated to remain between 15° and 15.5°C. After one hour it was removed, and after thoroughly drying, weighed, using another pycnometer as a counterpoise.

Weight of pycnometer	Counterpoise -	1.4900 grams
Weight of pycnometer + H_2O	Counterpoise -	6.8908 "
Weight of H_2O (15 + 15.5°C)		5.4008 "

After thoroughly drying the pycnometer again, it was filled with purified, filtered oil and brought into the water bath. After one hour it was removed, dried and weighed.

Weight of pycnometer + oil	Counterpoise +	6.4800 grams
Weight of Oil (15° - 15.5°C)		4.9900 "

$$\text{Density}(15^\circ - 15.5^\circ C) = \frac{\text{Oil weight}}{\text{water weight}} = \frac{4.9900}{5.4008} = .9239$$

Index of Refraction:- The Abbe refractometer was used in all determinations. First, it was carefully adjusted to give the standard reading with pure redistilled water at 15°. Five readings were obtained with 5 different drops of water, the readings varying from 1.3333 to 1.3335. The instrument was thoroughly dried and then the index of refraction of a sample of olive oil determined. This was found to be 1.4697 and 1.4698. After washing carefully with alcohol and ether and drying, a test was made on the oil. Four readings were taken and were found to check closely.

Index of Refraction at 15°C. (Abbe)	
Water(redistilled)	1.3333 - 5
Olive Oil	1.4697 - 8
Oil	1.4761 - 2

The oil was perfectly clear having been filtered twice before using.

Effect of Heat:- Upon warming about 1.5 cc of the oil in a small flame, it was observed that it did not decompose up to 100°C.

Then it was heated more strongly and finally up to 350°C when it appeared to grow dark. However, as yet no charring or offensive odor was detected. Upon spreading a thin layer of this resulting oil over a flat surface, it was found that the drying power was now increased materially.

We have thus far determined most of the chemical and physical constants of our oil, and it would be wellnow to place these in some sort of a table which will enable us to classify or group the oil. The classification or grouping with which it shall be compared, is that given by Lewkowitsch thruout his text. The saponification value of our oil is about that of the average oils, 193 - 194. is does not indicate any very great differentiation between the oil and oils of the drying, semi-drying etc. groups, but to a certain extent indicates that the fatty acids present are of a certain group. This will be discussed later under fatty acids. However, if we study the iodine values, we find that oils belonging to the "drying oil" class have values of 110 to 206 with most of them about 135-150. Again, those of the "semi-drying oil" class have values lying between 100 and 125, for the "Cotton Seed" group; 70 to 110 for those of "Rape oil" group. In the "non-drying oil" class the iodine value varies from 71 to 116, in the "Castor oil" group, however the iodine values lie between 83 and 142. However, it will be noticed that for the non-drying oils having iodine values 71 to 116, most of them lie around 85 and the saponification number around 195. This does not conform to our oil as much as the figures of the "Castor oil" group, iodine value 83 - 96, saponification value 183 - 190, as compared with iodine value 90, saponification value 193 for our oil.

Upon further examination, the specific gravity .9239 at 15.5°C,

is found to conform to the specific gravity of the oils found in the "Cotton seed" group, .9200 to .9250 at 15.5°C. The specific gravity of oils in the "Castor oil" group is between .9500 and .9600, much too high for our oil. However, it is true that we find both, grape seed oil and castor oil, classed as either semi-drying oils or non-drying oils according to Lewkowitsch. In his text of 1909 both are classed as non-drying oils. In his hand book, "Labroatory Companion to Fats and Oils Industries(1901)," they are classed as semi-drying oils. From this classification I would say that the oil from the seeds of the fruit of Ampelopsis Quinquefolia resembles the oils of the "Castor oil" group and should be put, if rincinolein is found present, in the "Castor oil" group. If this compound is not present, that is if ricinoleic acid is absent, I would classify the oil under non-drying oils, without putting it in the group. However, I will say that ricinoleic acid was determined and hence the oil belongs in the "Castor oil" group(see fatty acids). Upon comparing the index of refraction it will be observed that this to is concordant with those of the semi-drying oils.

Classification of Oils

Oil	Saponification value	Iodine value	Specific Gravity- 15.5	Index of Refraction	°C
Drying oils					
Linseed	192-195	173-201	.931-4	1.4835	
Hempseed	192.5	148	.925-8	1.4780	
Stillingia	210	160	.9432	1.4825	22.5
Millet Seed	183.5	130.4	.924-5	1.4611	60.
Sunflower	193.5	110.	------- -	------	
Pine Nut	192.8	101.3	------	1.4769	
Poppy Seed	195	133-143	.924-7	1.4760	
Madia	192.8	118.5	.9285	-------	
Manihot	188.6	137	------	------	

Classification of Oils (cont-)

Oil	Saponification value	Iodine value	Specific Gravity - 15.5	Index of Refraction	°C
Semi-drying Oils					
Cotton Seed oil group					
Daphne	196.5	126	.-----	------	
Melon Seed	193.3	101.5	.-----	--------	
Cotton Seed	193-195	108-110	.922-5	1.4743	15.0
Anis Seed	178.3	105.3	.------	-------	
Croton	210-215	102-104	.9500	1.4781	26
Curcas Nut	193.2	98-110	.9204	1.4631	25
Beech Nut	193.5	104-111	.920-2	--- -	
Rape oil group					
Garden cress	178-183	109-139	.920-2	--------	
Raddish seed	173-179	93-96	.9175	------ --	
Rape	170-178	94-102	.913-6	1.4720	15
Jamba	172.3	95.4	.9154		
Ravison	179	101-122	.9183		
Non-drying oils					
Quince	181.8	113.0	.9220	1.4729	
Cherry kernel	194.	110-114	.9234	------	
Almond	191.	93-97	.917-9	1.4555	
Tea Seed	194.	- --- -	.917-9	------	
Olive kernel	183.	87.4	.918-9	1.4682	25
Peach kernel	192.5	93-109	.918-9	------	
Hazel Nut	192.	83-90	.914-7	------	
Staff tree	223.5	86.7	-----	------	
Castor oil Group					
Grape Seed	178.5-190	96-142	.9350	1.4713	
Ampelopsis Quinquefolia	193-194	90-90.5	.9239	1.4762	
Castor	183-186	83-90	.960-7	1.4799	

Examination of Fatty Acids:- Separation of liquid- 2.6824 grams of oil - saponification number of which was 193 and iodine number 90- were saponified by boiling with 50 cc of caustic potash solution as in the determination of saponification number. The

saponification was carried out in a flask and the boiling continued for 5 hours. The soap was then diluted with 100 cc of water and the solution boiled in a porcelain dish until all the alcohol was evaporated off. Instead of preparing the free acids, the method of Gusserow* was followed from here on for the separation of the liquid and solid fatty adids. Gusserows method is based on the solubility of the lead salts of the liquid fatty acids(oleic, linolic, and linolenic) in ether, the lead salts of the higher solid fatty acids, steanic and palmitic, being practically insoluble in that menstrum, as will be gathered from the following table:-

Lead salt of	100 cc ether dissolve grams	Observer
Palmitic acid	0.0184	Lidoff*
Stearic acid	0.0148	Lidoff
Mixed stearic and palmitic acids(I. val.0)	0.0150(at 25°C)	Twitchell*

Hence let it be understood that this method does not yield strictly accurate results. Lewkowitsch also has shown that a complete separation of the liquid from the solid acids cannot be thus affected. Also, it must be borne* chiefly separation of solid and liquid acids which is not tantamount to a separation of saturated from unsaturated fatty acids, since the lead salt of erucic acid as also that of isooleic acid, is sparingly soluble in cold ether, while the method for determining arachidic acid is based on the solubility in ether of the lead soap. Therefore, we can say that we have used the method as outlined by Lewkowitsch in his, "Chemical Technology and Analysis of Oils, Fats and Waxes, Vol. 1-1909."

*Liebig's Annalen, 27(1828), 153
*Berichte, 26 ref. p. 97
*Jour, Soc. Chem. Ind.1895,515
* in mind that the Gusserow method effects

The alcohol free solution was then neutralized with acetic acid to phenolphthalein. Next 30 cc of a 10% lead acetate solution are diluted with 150 cc of water, brought to the boiling point, and gradually run into the soap solution with constant shaking, whereby the separating lead soap is made to adhere to the side of the flask when the solution becomes cold. The flask containing the lead soap was completely filled with hot water, and then allowed to cool. When the liquid has become clear it is poured off thru a filter. All the ppt. was found to adhere to the flask.Enough boiling water was added to fill it completely, the solution allowed to cool to room temperature and then filtered. This was repeated twice in all and the remaining drops of water were removed with a filter paper. Immediately 150 cc of ether were added to the lead salts, and the flask corked and shaken repeatedly, thus allowing the lead salts to disintegrate. It was then attached to a reflux condenser and heated on a water bath for one hour. A fine ppt. remained at the bottom of the flask and after cooling to room temperature the liquid was filtered, the ppt. being brought on the filter paper by washing it on with ether using 30 cc portions, repeating 4 times. The ethereal solution was poured into a separatory funnel and shaken with a mixture of one part HCl and 4 parts water, in order to decompose the salts. The ether takes up the free fatty acids, the lead chloride separating. The lead chloride and mineral acid solution was drawn off and the ethereal solution was washed, first with a dilute acid, HCl, solution and then 3times with distilled water, until the wash water was free from acid to litmus. The ethereal solution was then run thru a plaited filter into a tared erlynmeyer flask. The ether was evaporated until a volume of about 60 cc remained. A cork, which was grooved around the circumference with

"V" grooves made by cutting into the cork with a knife, was then inserted and the glass tube running thru the center attached to a CO_2 generator. The CO_2 was run thru a drying bottle containing $CaCl_2$ and lime saturated with CO_2. The flask was finally immersed in boiling water and kept therein for one hour when the resulting oil appeared to be perfectly clear. It was then removed, dried and placed in a vacuum desicator and kept under high vacuum for one-half hour, when the temperature in the vacuum read the same as that of the room. The desicator was then filled with air and the flask removed and weighed. The liquid acids were perfectly clear and fluid and of a yellow sparkling color.

Weight of flask + liquid acids	45.3882	grams
Weight of flask	43.1127	"
Weight of liquid fatty acids	2.2755	"

Equivalent to (2.2755÷ 2.6824)100 = 84% of oil. The lead salts of the solid fatty acids, which remained on the filter paper were washed into a separating funnel with ether and the acids liberated as above described. After drying and cooling as above also under an atmosphere of CO_2 we obtained a little substance, which appeared to be almost transparent and of crystalline form the latter resembling plates. However, so little was obtained that nothing could be done with it. We take up the work on solid fatty acids a little later again.

Weight of dish + solid fatty acids	33.8466	grams
Weight of dish	33.8050	"
Weight of solid fatty acids	.0416	"

Equivalent to (.0416 ÷ 2.6824)100 =1.6% of oil

This shows that the total percent of mixed fatty acids is 85.6% of the oil.

Iodine Number of Liquid Fatty Acids as obtained by Gusserow's Method*:- The method used was that of Hanus as described in Shermans, "Methods of Organic Analysis." This is the same as that used for determing the iodine value of the oil.

#1		#2
62.7632 grams	Weight of flask	62.7800 grams
63.1055 "	Weight of flask + sample	63.0232 "
.3423 "	Weight of sample	.2432 "
36.07	Blank requires cc $Na_2S_2O_3$	36.20
12.38	cc $Na_2S_2O_3$ required to neutralize	15.10
23.69	equivalent number of cc I used up	21.10
82.3	Iodine Value	103.2

$$\frac{23.69 \text{ x } .12685 \text{ x } .0938 \text{ x } 100}{.3423} = 82.3$$

The results were obtained under different conditions of time. #1 was allowed to stand but one-half hour. #2 was allowed to stand 4 hours. From this it appears that #1 was not allowed time enough for complete reaction to take place.

Iodine value of Solid Fatty Acids obtained above:- Here again we used the method of Hanus.

Weight of dish + solid fatty adids		33.8466 grams
Weight of dish		33.8087 "
Weight of fatty acids		.0379 "
Blank requires	28.75 cc	$Na_2S_2O_3$ .0938 N
Sample requires	27.05 cc	$Na_2S_2O_3$ .0938 N
Hence	1.79 cc	I_2 were absorbed

$$\therefore \frac{1.70 \text{ x } .12685 \text{ x } .0938 \text{ x } 100}{.0379} = 53.4 \text{ Iodine Number}$$

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*Later we obtained the iodine number of the liquid Fatty acids according to Tortelli and Ruggari.

This appears to be a rather high iodine number for solid fatty acids, but in a later discussion a proposed explanation is offered. (See examination of Solid fatty acids, page 45). The fatty acids themselves, appear as colorless plates, had somewhat of an odor, faintly disagreeable, and were greasy to the touch. Just for a matter of comparison (as will be shown later) let us calculate the iodine number of the mixed fatty acids.

2.2755 grams weight of liquid fatty acids
.0416 " " " solid " "
2.3171 " " " total " "

2.3171 ÷ 2.6824 = 86.% of oil

.2432 grams liquid fatty acids used for I number detection
.0379 " solid " " " " " " "
.2811 " total " " " " " " "

21.10 cc $Na_2S_2O_3$ used for liquid acids
1.70 cc " " " solid "
22.80 cc " " " total "

Hence $$\frac{22.80 \times .12685 \times .0938 \times 100}{.2811} = 96.5$$

Iodine Number (calculated) of total fatty acids 96.5

Neutralization Number:- Of liquid fatty acids:- The neutralization of potassium hydrate required to saturate one gram of the mixed fatty acids. One may infer from this definition that this value was meant for a mixture of solid and liquid acids only, but it is true that both of these consist of mixed fatty acids, hence the neutralization number means the same for liquid, solid or mixed fatty acids. Since the saponification of our oil is 193, we need expect to find but little, if any, of the lower, volatile fatty acids. However, this will be discussed later in the chapter. For

the liquid fatty acids the determination was carried out as follows: 1.5995 grams of the acids were weighed out into an Erlynmeyer flask and 25 cc of pure methyl alcohol added. This dissolved the oil completely. To another similar flask 25 cc of pure methyl alcohol only were added. To each were added 5 drops of phenolphthalein and the resulting solutions titrated with .5155 normal alkali.

25 cc methyl alcohol blank required 0 cc alkali

25 cc " " acid solution required 10.60 cc

$$\frac{10.60 \times .5155 \times 56 \times 1000}{1000} = 306.5 \text{ mg - KOH}$$

Hence 306.5 mg KOH were required to neutralize 1.5995 grams liquid fatty acids or

$306.5 \div 1.5995 = 192.$ = neutralization value.

From the neutralization value thus found the mean molecular weight of the fatty acids can be calculated*, thus

Let M be the mean molecular weight of fatty acids.

n the number of grams of KOH found by exp. to neutralize 1 gram of fatty acids, then

$$M = \frac{56.1}{n} \text{, and therefore,}$$

$$M = \frac{56.1}{n} = \frac{56.1}{.192} = 292.$$

During all these examinations, we ran similar tests on olive oil and acids from olive oil. The results thus obtained for our oil, were obtained, therefore, under conditions which were satisfactory for obtaining concordant results for olive oil. Not only were the results for olive oil concordant, but they checked well with the results given in Lewkowitsch's tables. Thus the neutralization value of the mixed fatty acids obtained from olive oil was 214. This, however, included any water soluble acids which may be present in the oil. If we deduct the number of mg. of KOH required

*Lewkowitsch, Vol.1, page 414.

to neutralize the free fatty acid(as free in oil), we obtain about 208. Various tables give this value as 193 - 195. The iodine value of the liquid fatty acids of olive oil, as obtained by Gusserow's method, in our case was found to be 83, while Lewkowitsch gives it as 93 - 112, as determined of liquid fatty acids obtained according to Tortelli and Ruggeri. However, before attempting to do any classifing or to make any definite conclusions, it was decided to obtain more oil and carry out more carefully the same determinations besides some additional ones.

Second extract of oil from seeds:- The extraction was carried out in exactly the same manner as that described on page 17, using 90 grams of seeds. The oil appeared, in general, as that previously obtained, in specific, reacted alkaline to the acid value test as applied to the former sample.

Saponification Number:- 2.9897 grams were saponified as described under saponification(page 18).

Time of saponification	3 hours	
Weight of flask + oil	46.9034 grams	
Weight of flask -	43.9137 "	
Weight of oil	2.9897 "	
Blank required	68.80 cc	.5165 acid.
Sample required	49.65 cc	.5165 " .

$$\frac{19.15 \text{ x } 56.16 \text{ x } .5165 \text{ x } 1000}{1000 \text{ x } 2.9897} = 186$$

The result is a little lower than that obtained before, but this is probably due to the presence of a slight amount of petroleum ether in the sample.

Iodine Number:- This was, likewise determined as previously described(page 19).

68.8960 grams	Weight of flask	63.0310 grams
69.1360 "	Weight of flask + oil	63.2772 "
.2400 "	Weight of oil	.2462 "
28.05	Blank requires cc $Na_2S_2O_3$ .0938 N	28.05
10.30	cc required to neutralize	10.16
17.75	cc Iodine used	17.89
88.	Iodine Value	86.

Here again our values are slightly lower than before, probably due to presence of trace of petroleum ether.

Preparation of Mixed Fatty Acids[*]:- About 18 grams of oil were saponified by boiling with 50 cc of caustic potash solution, sp. gr. 1.4, and 50 cc of alcohol in a flask provided with a reflux condenser. The resulting soap(after saponifying 12 hours) was dissolved in 650 cc of water and the solution boiled until all the alcohol was evaporated off. A portion of this solution was then taken, placed in a separatory funnel and ether added. To this mixture we added 50 cc of a solution of HCl(1:4) and shook thoroughly. The acid solution and $PbCl_2$ were then drawn off and the ether solution treated with 50 cc more of HCl solution(1:8). After drawing off as completely as possible the water solution, the ether was washed with distilled water until the water was free from acid to methyl orange. Thus all water soluble acids were to be found in the acid(water) solution. The ether extract was concentrated and when but 30 cc were left, the remaining ether was evaporated off under an atmosphere of CO_2 . The resulting acids were of a clear yellow color.

Iodine Number of Mixed Fatty Acids:- This was determined in

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[*] Lewkowitsch,-Vol.1, page 88.

the same manner as that of the oil.

#1		#2
68.6155 grams	Weight of flask	62.7669 grams
68.8532 "	Weight of flask + oil	62.9660 "
.2377 "	Weight of M. F. A.	.1991 "
27.67	Blank requires cc $Na_2S_2O_3$ .938 N	27.67
9.73	cc required to neutralize	10.56
17.94	cc I_2 used up	17.11
90.	Iodine Number	102.

The average of these two determinations is 96, and it will now be remembered that this was the calculated value found on page 31.

Neutralization value of mixed fatty acids:- The method used was that employed previously for this determination of liquid fatty acids.

Weight of flask	41.9607 grams
Weight of flask + acids	43.7193 "
Weight of total fatty acids	1.7586 "

Sample consists of mixed fatty acids + unsaponifiable matter, altho the latter in only small quantity.

Blank requires	0.00 cc NaOH
Sample requires	12.10 cc.5281NaOH

$$\frac{12.10 \times .5281 \times 56.1 \times 1000}{1000} = 358.48 \text{ mg KOH}$$

Therefore 358.48 + 1.7586 = 203.4 mg KOH per gram mixed acids+unsap.

Mean molecular weight equals $\frac{56.1}{.203} = 275.$

Acetyl value of mixed fatty acids:- The acetyl value indicates the number of milligrams of caustic potash required for the neutralization of the acetic acid obtained on saponifying one gram of an acetylated oil, fat, etc. The determination is based on the fact

that glycerides containing hydroxylated fatty acids assimulate, on heating with acetic anhydride, one or more acetyl groups, according to whether the fatty acids contain one or more alcoholic hydroxyl groups. The determination was carried out as proposed by Lewkowitsch*;- .4150 grams of mixed fatty acids were boiled with about one gram of acetic anhydride for two hours in a round bottom flask attached to a reflux condenser. The residue was washed onto a filter paper and washed until free from acid(litmus paper). The resulting residue was boiled with alcoholic potash in the same way as performing a saponification, and after diluting with water the alcohol was evaporated off. Then the acetic acid was determined by means of the distillation process. Dilute H_2SO_4 was added to the soap solution and the mixture was boiled the steam vapors carrying over the acetic acid vapors. About 250 cc of water were distilled. The distillate was titrated with .5281 N NaOH.

Weight of flask	57.4365 grams
Weight of flask + sample	57.8515 "
Weight of sample	.4150 "

.6 cc of .5281 N alkali was used to neutralize the distilled acid.

.6 x .5281 = X .1 or is equivalent to 3.17 cc N/.1 alkali

$$(3.17 \times 5.61) \div .415 = 42.85$$

Acetyl value	42.85

This, however, is the acetyl value of the mixed fatty acids. Not having any oil available for use, it may be of some interest to calculate roughly what may be the acetyl value of the oil. We have found that 86% of the oil is what we called insoluble fatty acid. Since the oil upon which we figured this percent contains the unsaponifiable matter and this unsaponifiable matter, in part

* Jour. Soc. Chem. Ind. 1897, 503

at least, is present in the fatty acid, we can say approximately that the acetyl value of the oil is $\frac{84}{100}$ x 42.85 or 36. Of course any alcohols present, were saponified. Also if any volatile acids were present in the mixed fatty acids, they naturally were distilled over and would increase the acetyl value. It was very unfortunate that we did not determine the latter, it being completely overlooked until too late, when no more acid was available. However, roughly the acetyl value of the oil may be considered as 36.

Separation of Liquid and Solid Fatty acids-- Method of Tortelli and Ruggeri*-- The method of Tortelli and Ruggeri for the separation of liquid and solid fatty acids, combines the advantageous features of several methods outlined in Lewkowitsch and gives as a result, liquid fatty acids having the highest iodine value found hitherto, and which for that reason must be accepted as being the nearest to the true ones. An examination, to determine the individual fatty acids was made, after the separation, both of the liquid and solid acids obtained. The operation was carried out as follows:- About 16 grams of oil were saponified with 15 cc of an aqueous 50 % solution of KOH and 45 cc of 95 % alcohol. The excess of KOH was neutralized with acetic acid, phenophthalein being used as an indicator. In a half-liter flask 300 cc of 7 percent lead acetate solution was poured into it in a thin stream, with constant shaking. The flask was immersed in cold water and kept therein for 15 minutes, shaking constantly. The supernatant liquid now being clear was poured off and the lead soap washed three times with 200 cc of warm(not boiling) water cooling each time before filtering. To the lead soap, which was dried with

*Lewkowitsch, Vol,1,page 447.

filter paper, 220 cc of ether were added. the mass thoroughly shaken and then warmed in a water bath for 20 minutes until the ether just started to boil. The flask was then immersed in ice-water, kept at 8° - 10° and kept therein for 2 hours. The liquid was then filtered thru a plaited filter into a 200 cc flask, the flask filled with ether and, left standing in running water for 14 hours. A little ppt. yellow in color separated. After filtering a portion of the ethereal solution was decomposed with 150 cc of 20% HCl. This was repeated and the ether washed and evaporated to 50 cc. The ppts. were combined and treated as under, "Solid Fatty Acids."

Liquid Fatty Acids:- The 50 cc of ether solution of acid were put into a 100 cc flask and the ether evaporated under an atmosphere of CO_2. This was done as described, on a water bath. The iodine number of the acids thus obtained was found to be within 125 and 130. The ethereal solution of lead salts remaining was treated as follows:- The ether was removed under an atmosphere of CO_2, leaving a viscous syurpy red mass of lead salts of quite pleasant odor. These did not solidify in running water but if placed in water a little below 0°C solidified to a red solid. To the lead salts, 200 cc of petroleum ether(low boiling) were added and the mixture boiled for two hours under a reflux condenser. The solution was then placed in a water bath for 1 hour(water bath at 0 C). After carefully filtering, a white gumy ppt. similar to the ppt. obtained on treating the liquid fatty acids of castor oil in this way, was obtained. This was carefully washed with pet. ether and after drying was found to melt at 97° - 98°C. The total precipitate amounted to about 1 gram and for this reason it was impossible to carry out any further tests. However, it might be said that an indication of rincinoleic acid presents itself. We carried on simultaneously,

determinations of castor oil thus attempting to keep to satisfactory conditions at least to a great extent. From castor oil we obtained the rincinoleic acid of iodine number 83.6 and acetyl value. The lead salt of the ricinoleic acid of castor oil melted at 99°C. I am sorry to say we did not have enough material to carry out confirmatory tests, but as far as physical appearance of the lead salt was concerned, I would say that the presence of a small amount of rincinoleic acid is indicated.

The petroleum ether filtrate was treated thus:- First, after standing two days, it was noticed that it changed from an orange color to a dark yellow. This was then treated with a solution of HCl(1:4) and then some ether was added. After separating a sticky greasy ppt. remained floating on the water and it would not dissolve in either pet. ether or ether. Both ether and pet. ether were added to take up all fatty acids, one possibly dissolving an acid, the other would not. Natural dihydroxystearic acid might be suggested for the residue observed, altho no tests were carried out. The ether extract was treated as usual, being evaporated off under an atmosphere of CO_2.

Iodine numbers obtained:- Determined as usual. First portion immediately after cooling to room temperature by holding cold cloth around flask.

Weight of flask	66.6282 grams
Weight of flask + sample	66.7213 "
Weight of sample	.0931 "
Blank requires	9.23 cc thio. .1852 normal
Sample requires	4.25 cc to neutralize
Iodine Number	126.

A second sample was carried out a little later.

Weight of flask	68.6652 grams	
Weight of flask + sample	68.8162 "	Time 4 hours
Weight of sample	.1510 "	
Blank requires	9.23 cc of .1852 normal	
Sample requires	3.20 cc to neutralize	
Iodine Number	93.	

A third was carried out allowing it to react with I_2 all night.

Weight of flask	68.3638 grams
Weight of flask + sample	68.8266 "
Weight of sample	.1628
Blank requires	9.25 cc thio. .1852 normal
Sample requires	3.10 cc to neutralize
Iodine Number	88.7

From this it appears that as the sample was allowed to stand it was rapidly oxidizing and for this reason I think the value 126 is the one nearest the true one. This iodine number, of course, is the value of the liquid fatty acids whose lead salts are soluble in ether and petroleum ether. The liquid fatty acids, like the oil, give the elaedin reaction forming a hard white solid and turning the remaining liquid dark brown. Upon treating a portion of the liquid fatty acids with conc. H_2SO_4 a reddish brown compound is formed, soluble in alcohol(warm) and when treated with water in H_2SO_4 solution gives a white colloidal ppt. and an oil that resembles very much the oil left after similarly treating ricinoleic acid. On letting the liquid fatty acids stand a white solid acid separates.

Neutralization number of Liquid fatty acids(leads salts of shich are soluble in pet. ether and ether).

Weight of liquid acids used 2.4300 grams

cc KOH required 22.35 .3310 normal

$$\frac{22.35 \times .3310 \times 56}{2.43} = 170$$

Mean molecular weight 330.

Low result may be due partially to presence of trace of pet. ether.

Oxidation of Liquid Fatty Acids.- A portion of the liquid fatty acids obtained, according to the Tortelli and Ruggeri method, and further that portion of liquid acids whose lead salts are soluble in ether and pet. ether, was examined for individual adids according to the oxidation method. A general rule, stated by Hazura and Guissner, can be applied to the oxidation of unsaturated acids. Of course one must bear in mind that liquid and solid fatty acids are not tantamount to unsaturated and saturated acids, but that to a certain extent some of the one may be found in the other. The rule may be stated as follows:- All unsaturated fatty acids, when oxidized with $K MnO_4$ in alkaline solution, have as many hydroxyl groups added as there are unsaturated valencies in the molecule, and yield as oxidation products saturated hydrosylated acids containing the same number of carbon atoms. Bearing this in mind and referring to the table given in Lewkowitsch Vol. 1, page 170, we carried out the oxidation and examination as follows:- About 2.5 grams of the acids were dissolved in alcohol and neutralized with KOH. Then the alcohol was boiled off and the soaps dissolved in 200 cc of water. 200 ccof 1 1/2% $KMnO_4$ solution were added in a thin stream and the mixture constantly agitated. The solution was allowed to stand 10 minutes and then SO_2 bubbled into the solution until all the MnO_2 was dissolved and the solution reacted acid.

1- Precipitate - yellowish white - The mixture(water solution) was washed with 50 cc of ether to remove all unoxidized oils. Then 300 cc of ether were added to the remaining solution and this allowed to stand 20 minutes, when the ether was removed and the water extract treated with a fresh portion of ether. This was repeated 3 times using 300 cc each time. The ether was then distilled off and when finally but 50 cc remained, it(the 50 cc) was poured into a crystallizing dish and allowed to crystallize. Only a few crystals were obtained and after washing with ether and recrystallizing twice, we found them to melt(not very sharply) at 130 - 134°. They did not have any definite crystalline form when examined under the microscope, and thus it is evident that dihydroxystearic acid may be present but further evidence is necessary. After boiling out the ether of the water solution, the solution was diluted to 500 cc and boiled for 20 minutes, filtered hot and then boiled down to 100 cc. The ppt. was treated again with 500 cc of H_2O boiled and again filtered. This was repeated a third time. Finally the complete volume of water was boiled down to 250 cc and allowed to crystallize at ordinary temperature. After 2 days long sliky needles separated, which after washing with cold water, then ether and dried melted at 170°C. The total weight obtained was .0500 grams. This would indicate the presence of sativic acid, but here again further evidence is necessary.

2- The acid filtrate-- This was neutralized with KOH, boiled down to 150 cc, and then acidulated with H_2SO_4. After drying what little ppt. was obtained, it was found that, after washing the dried powder with ether, there was too little left too work with. Hence the investigation was not carried any further.

Separation of Individual Liquid Fatty Acids.(Lewkowitsch)

<table>
<tr><td colspan="5">Liquid Fatty Acids(lead salts):- Add low boiling pet. ether boil 2hr</td></tr>
<tr><td>Residue:- lead ricinoleate</td><td colspan="4">Filtrate:- contains fatty acids whose lead salts are are soluble in pet. ether. Evap. all pet. ether and decompose with HCl, add ether.</td></tr>
<tr><td rowspan="5">Determine melting pt. and other tests - I_2 no. acetyl no. etc.</td><td colspan="4">Ether extract:- Contains all liquid fatty acids. Evaporate ether. Determine constants and then neutralize remainder with KOH</td></tr>
<tr><td colspan="4">K salts in H_2O:- Make solution slightly alkaline and oxidize with $KMnO_4$.</td></tr>
<tr><td colspan="2">Ppt. A. Dihydroxystearic and sativic acid. Extract with large volume of ether.</td><td colspan="2">Filtrate B- Isolinusic and linusic acid conc.- Make acid H_2SO_4.</td></tr>
<tr><td rowspan="2">Filtrate Dihydroxy-stearic acid Identify</td><td rowspan="2">Residue Mixture with more sativic acid. Boil out with water and crystalize Identify</td><td colspan="2">Ppt.- Isolinusic and linusic acid. Dry and extract with ether.</td></tr>
<tr><td>Residue:- Isolinusic and linusic acid. Crystallize from alc.
Still a mixture remains.
Recrystallize from water
1-st crop Linusic acid (less soluble in water).
2-nd crop Isolinusic acid.</td><td>Filtrate:- secondary oxidation products.</td></tr>
</table>

Of course this table is made on the assumption that only the 3 acids, oleic, linoleic, linolenic were present. We had so little material that we attempted only this separation.

Examination of Solid Fatty Acids:- The lead salts left after the ether treatment in the method of Tortelli and Ruggeri are the lead salts, partially at least, of the solid fatty acids. These are insoluble in ether, to a great extent, and hence are separated from the more soluble ones. After washing the salts, with ether, into a separatory funnel, a solution of HCl(1:4) was added liberating the acids and ppt. $PbCl_2$. The acids were taken up by the ether and the ether extract drawn off and evaporated leaving the solid fatty acids. The acids crystalluze in plates and are white or just pale yellowish white(cream color). They are soluble in alcohol (cold). The melting point of the acids as deposited from ether is 41°C. The total weight of ppt.(1 gram) was washed with water into a round bottom flask and distilled with steam. The filtrate containing 200 cc of water, required .65 cc of NaOH - .1123 N to neutralize to methyl orange. This indicates the presence of an acid volatile with steam or at least one which is volatile to a certain extent, and whose lead salt is insoluble in water and ether. By comparing this property, together with its meltingpoint, with a table given in Lewkowitsch(Vol. 1 page 95) we may suspect the presence of any of 3 combinations;-1 - lauric and myristic, 2 - lauric and palmitic, 3- lauric and stearic.

Melting Points of Mixtures of Lauric Acid with Myristic, Palmitic and Stearic Acids.

Lauric Acid	Myristic Acid		Palmitic Acid		Stearic Acid	
	Percent	M. P.	Percent	M. P.	Percent	M. P.
100	0	43.6°C	0	43.6	0	43.6
90	10	41.3	10	41.5	10	41.5
80	20	38.5	20	37.1	20	38.5
70	30	35.1	30	38.3	30	43.4
60	40	36.7	40	40.0	40	50.8
50	50	37.4	50	47.0	50	55.8
40	60	43.0	60	51.2	60	59.0
30	70	46.7	70	54.5	70	62.0

Now, we recrystallized our acids from alcohol and after 5 crystallizations obtained 2 sets of crystals giving definite melting points, one set melting at 45° C, the other 67° C: and crystals between these two melting at 54 - 60° C. Hence we could suggest for further imvestigation, that one might look for palmitic and stearic acid and that lauric acid or myristic acid is the volatile constituent: with more emphasis on lauric acid because myristic acid combined with palmitic and stearic acids gives melting points varying from 48°C to 69°C a little too high for that obtained.

However, we have not discussed the acid or acids, as the case may be melting at 45°C. In the first place, we determined the iodine number of the solid fatty acids as obtained form the ether extract and obtained the value given below.

	grams	
Weight of flask	66.6325	Time allowed
Weight of flask + sample	66.6760	to stand -
Weight of sample	.0435	12 hours,
Blank requires	9.25 cc	thio(.1852)
cc thio required to neutralize	8.15 cc	"
Iodine number	59.+	

It will be observed, if we look back, that the iodine number of the solid fatty acids obtained according to Gusserow's method, is 53.+ It was at first thought that this high number was due to liquid acids left in the solid(due to incomplete separation). However, we obtain a value here which, according to the method of Torelli and Ruggeri ought to be considerably higher tha the one obtained, because in this method the separation is not as effective as that of Gusserow. The conclusion drawn, therefore, is that the solid acid, melting at 45°C and crystallizing in transparent plates from ether, and being a constituent of the mixed solid fatty acids, which have an iodine value of 56(average), is isooleic acid.

Upon examining further it is noticed that the lead salt of isooleic acid is considerably less soluble in ether than in lead oleate, this taken from Lewkowitsch, and which would explain why isooleic acid might be expected in the solid acids. We also found these transparent crystals to dissolve easily in alcohol. Of course, a great deal more investigation is necessary to prove definitely the actual presence of all the acids here enumerated. We worked with very small quantities and no doubt errors occurred. However, special attention should be given to those acids, here suggested as being qualitatively determined. If one looks over the literature he will observe numerous acids melting between 35 and 55°C, Elaidic acid 44.5°C, Erucic acid 33 - 34°, Brassidic acid 65°C, Isoerucic acid 54 - 56°C, all of which may be the acid here reported as isooleic, our acid in that case, being either contaminated or an "alloy" or "eutectic" compound. However, it stands that the acid obtained, was one crystallizing in transparent crystals and melting at 45°C.

SUMMARY

We have classified the oil obtained from the seeds of the Ampelopsis Quinquefolia(Virginia Creeper) with castor oil, grape seed oil and to a certain extent with croton oil. How this grouping may fit the oil, will be further discussed upon data obtained from the examination of its fatty acids. Accordingly, then let us enumerate the constituents, as given in various papers, of the 3 oils in this group, and also those of the Virginia Creeper seed as indicated from our work. We will notice if we look at the table, that the neutralization values of the mixed fatty acids of all four of these oils lie quite close together. Also if we observe the percent of liquid fatty acids in the oil of Virginia Creeper, we

see that almost all of the fatty acid is liquid fatty acids, 86%.

Constituents of Oils of Castor Oil Group

Castor oil		Grape Seed oil		Croton oil		Ampelopsis Quinquefolia	
Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid
Tiglic Dihydroxy- stearic Stearic	Ricinoleic Linolic? Isoricinoleic	Stearic Palmitic	Linolic Oleic Ricinoleic Erucic?	Tiglic Stearic Palmitic Myristic Lauric Caproic Butyric Acetic	higher homologues of oleic.	Stearic Palmitic Lauric or Myristic Dihydroxy- stearic? Isooleic.	ricinoleic Oleic Linolic

The neutralization value of these liquid fatty acids is 192 which agrees quite well with the neutralization value of the mixed fatty acids of castor oil. Therefore, it would be reasonable, at least, to expect to find acids of similar nature as those of castor oil in the Virginia Creeper. And this is quite the case. Castor oil contains as liquid fatty acid ricinoleic, linolic and isoricinoleic acid. Ampelopsis Quinquefolia contains, ricinoleic, oleic, and linolic acid. It might be of some importance to point out here that the neutralization value of the liquid fatty acids, whose lead salts are soluble in ether and pet. ether, is 170 and this indicates a mean molecular weight of 330. From the table given in Lewkowitsch this would be an indication of erucic acid (M. M. W. 338) and since this acid has properties so similar to oleic acid, a search for it may prove to be profitable. However, we did not carry out any determinations, falling short of material. Again the iodine numbers of all four agree quite reasonably. the iodine numbers of the liquid fatty acids seem to indicate quite clearly that castor oil does contain to a certain extent linolic acid, and that grape seed oil contains much more, while Ampelopsis Quinquefolia contains some

and experimental facts as here found, indicate at least, the presence of linolic acid.

Not much can be said about the solid fatty acids, except that in the oil of Ampelopsis Quinquefolia only 1.6% of the total fatty acids are solid fatty acids. These consist chiefly of isooleic acid, and stearic acid, together with some lauric or myristic acid with possible presence of dihydroxystearic. These also conform quite well with those of the other oils in this group.

The fruit Ampelopsis Quinquefolia was examined, the extraction being made largely by the method proposed by Parsons. We found 12.96% of combined sugars, comprising 3% dextrose, 7.74% levulose , 2.22% sucrose. Determining the organic acids, gave the following approximation: oxalic acid 1.21%, (air dry fruit), tartaric acid and citric acid .58%. The seeds contained 25% oil, represention 15% of the whole fruit. The oil is yellow in color tasteless, odorless, semi-drying, drying power increased on heating. From the analytical data obtained for the oil we have classed it with the Castor Oil Group comprising Castor Oil and Grape Seed Oil.